



## Original article

Food for honeybees? Pollinators and seed set of *Anthyllis barba-jovis* L. (Fabaceae) in arid coastal areas of the Mediterranean basin

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## ABSTRACT

Abundance and diversity of insect pollinators are declining in many ecosystems worldwide. The abundance and diversity of wild and managed bees are related to the availability of continuous floral resources. In particular, in Mediterranean basin countries, the presence of wildflower spots enhances the establishment of social Apoidea, since coastal regions are usually characterized by pollen and nectar shortage in early spring and late summer. *Anthyllis barba-jovis* produces both nectar and pollen as important food source for bees helping them to overcome early spring period food shortage. We investigated flowering, seed set, and pollinator diversity of *A. barba-jovis* in arid coastal environments of the Mediterranean basin. Pollinator abundance reached a maximum in early April. Honeybees were the most common pollinators followed by bumblebees and solitary bees. Plants prevented from entomophilous pollination showed inbreeding depression with a strong decrease in seed-set. To the best of our knowledge, this is the first report on pollination ecology of *A. barba-jovis*.

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## 1. Introduction

Insect pollinators' abundance and diversity are declining in many ecosystems worldwide (Bommarco et al., 2012). This seems mostly due to large-scale agricultural intensification (Decourtye et al., 2010), since the loss of semi-natural habitats and overuse of agrochemicals have detrimental effects on nesting and foraging activities of pollinators (Wratten et al., 2012; Rollin et al., 2016). The health of wild and managed honeybees is declining substantially over the last decade, both in Europe and USA (Becher et al., 2013). Huge losses of honeybee colonies have been observed worldwide, particularly in USA from 2006, when colony collapse disorder (CCD) was described (Oldroyd, 2007). CCD seems to be due to multiple stressors, such as occurrence of epidemiological factors affecting insect health (e.g. diseases and parasites) (Cox-Foster et al., 2007; Le Conte et al., 2010), overuse of pesticides and other agrochemicals (Becher et al., 2013), and loss of flower-rich plant communities associated with traditional agricultural

landscapes (Potts et al., 2010). The abundance and diversity of wild and managed bees are related to the availability of continuous floral resources (Wratten et al., 2012; Brittain et al., 2013; Nicholls and Altieri, 2013). In particular, in Mediterranean basin countries, the presence of wildflower spots enhances the establishment of social Apoidea, since coastal regions in the Mediterranean basin are usually characterized by pollen and nectar shortage in early spring (Canale et al., 2015) and late summer (Benelli et al., 2014).

*Anthyllis barba-jovis* L. (Fabaceae) is an evergreen shrub found in different habitats along the rocky cliffs of the western-central Mediterranean basin, including France, Italy, Croatia, Algeria and Tunisia (Morbidity et al., 2008). Even if *A. barba-jovis* is not included in the World Conservation Union Red List, it is a protected species at a national level in France (Danton and Baffray, 1995) and Croatia (Trinajstić, 1994). In Italy, this species is not listed in the Protected Flora, but it is considered to be in a risk category in seven of the nine regions where it is found (Morbidity et al., 2008).

Little has been reported about the biology and ecology of this species. Only pollen morphology (Díez and Ferguson, 1990) and seed germination ecology (Morbidity et al., 2008) have been studied. Despite this lack of information on this Mediterranean shrub, the genus *Anthyllis* is rich in species showing co-evolution towards mutualism with Apoidea pollinators (e.g. *Anthyllis hermanniae*, Yang et al., 2014; *Anthyllis vulneraria*, Navarro, 1996, 1999, 2000). We hypothesize that *A. barba-jovis* could be a pivotal source of pollen and nectar for Apoidea in arid coastal areas of the Mediter-

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ranean environment, helping them to overcome early spring periods characterized by pollen and nectar paucity. In this research, we investigated the early flowering of *A. barba-jovis* in an arid coastal area of Tuscany (Italy). Pollinator diversity and abundance were studied, in order to determine if this rustic species could be proposed to enhance floral diversity supporting Apoidea populations in agricultural areas of Mediterranean basin countries. The role of entomophily on seed-set was also tested in the field.

## 2. Materials and methods

### 2.1. Study area

Investigations were carried out during *A. barba-jovis* flowering (from early March to late May 2015) in a rocky arid environment of coastal Tuscany (i.e. 20 m a.s.l., Caligniaia, 43.464N, 10.342E, Livorno, Italy) (Fig. 1a). The climate is Mediterranean, with summers cooled by the sea breeze (mean temperature:  $26 \pm 6^\circ\text{C}$ ) and warm winters (rarely below  $4^\circ\text{C}$ ). Rainfalls were scarce (i.e.  $<500\text{ mm/year}$ ) and concentrated in early spring and autumn. The substrate is formed by calcareous rocks (i.e. sandstone from the “Macigno Formation”, Late Oligocene). The floristic association of the studied area was classified as *Crithmo-Staticetum*, frequently exposed to sea-salt spray during windy periods. This vegetation is rich of chaparral shrubs (e.g. *Cistus monspeliensis*, *Myrtus communis*, *Phillyrea angustifolia*, *Pistacia lentiscus* and *Rhamnus alaternus*), as well as coastal species, such as *Euphorbia pithyusa*, *Juniperus phoenicea*, *Juniperus oxycedrus* var. *macrocarpa*, *Helichrysum italicum* and *Senecio cineraria*. In this area, three homogeneous *A. barba-jovis* strips (i.e. length: 10 m, width: 2 m, parallel to the coastline) were selected. For each strip, 10 plants were marked and studied for flowering dynamics, insect pollinators' visits and seed-set experiments. No beekeeping activities were detected within a radius of 6 km.

### 2.2. Flowering and abundance of insect pollinators

Flowering dynamics was studied following the methods reported by Benelli et al., (2014). From early March to late May 2015, samplings were carried out every week (three replicates for each sampling) and the number of open inflorescence per plant was noted. Insects were captured, using an entomological net, during their foraging on *A. barba-jovis* flowers. From March 1<sup>st</sup> to May 30<sup>th</sup>, four samplings per month (one each week) were carried out. For each sample date, two observation periods were chosen: morning (from 11:00 to 12:00) and early afternoon (from 14:00 to 15:00) (Benelli et al., 2014; Canale et al., 2014). Collected specimens were kept separately in plastic test tubes with cork shavings imbued with ethyl acetate until they could be prepared for identification. Insects were dry mounted, observed using a binocular microscope (Leica ES2, Germany) and identified at a species level. A sample ranging from two to five specimens for each species was observed with an environmental scanning electron microscope (ESEM, hereafter) (FEI Quanta™ 200, Hillsboro, USA) to ensure the presence of *A. barba-jovis* pollen (Fig. 1b) on the insect body, thus allowing us to legitimate the species as pollen foragers on the plant (Canale et al., 2014). Voucher specimens of all species were stored in entomological boxes and kept at the Department of Agriculture, Food and Environment of the University of Pisa. During summer 2015, appreciable flowering of honeybee-visited flora was recorded by direct observation. Observations were conducted in a radius of 500 m from the plants of *A. barba-jovis* flower spots surveyed for abundance of insect pollinators (Canale et al., 2015; Benvenuti et al., 2016).

(a)



(b)



Fig. 1. (a) Plants of *Anthyllis barba-jovis* at the study site (20 a.s.l., Caligniaia, 43.464N, 10.342E, Livorno, Italy). (b) Scanning electron micrograph of pollen grains from a flower of *Anthyllis barba-jovis*.

### 2.3. Role of entomophily on seed-set

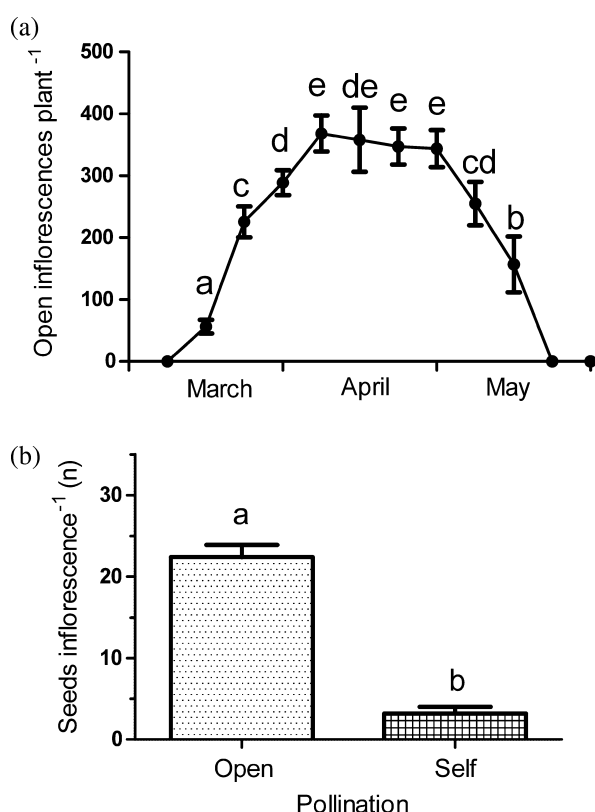
To establish the requirement for insect pollination for seed-set, some *A. barba-jovis* inflorescences were made inaccessible to visiting insects during March, April and May 2015. Following the methods described by Benelli et al., (2014), the buds of some inflorescences were “bagged” (BG) in pre-flowering with tulle mesh bags. Tulle is sufficiently fine to prevent insects from reaching flowers, but has a coarser weave (1.2 mm) over nylon or muslin (0.5–0.7 mm), allowing more airborne pollen to pass through, whilst still being insect-proof. Other *A. barba-jovis* inflorescences, the “open pollination” (OP) ones, were left open to flower-visiting insects. After senescence, 20 BG inflorescences were harvested from each of the three sub-plots (total: 60 BG inflorescences/season) and compared with 20 OP inflorescences per subplot (total: 60 OP inflorescences/season). The plant material from both treatments was collected and transferred to the University of Pisa laboratories. For each inflorescence, the number of seeds and their relative weight were noted.

## 2.4. Data analysis

*A. barba-jovis* flowering and seed-set data were checked for normal distribution, then analysed by JMP (1999) using one-way ANOVA. A probability level of  $P < 0.05$  was used for the significance of differences between values. Differences in the total abundance of insect pollinators were analysed using a weighted generalized linear model with two fixed factors:  $y = X\beta + \varepsilon$  where  $y$  is the vector of the observations (pollinator abundance),  $X$  is the incidence matrix,  $\beta$  is the vector of fixed effects (period and pollinator species) and  $\varepsilon$  is the vector of the random residual effects (Benelli, 2017). A probability level of  $P < 0.05$  was used for the significance of differences between values.

## 3. Results

*A. barba-jovis* flowering lasted from early March to late May. The number of open flowers varied significantly during the observation period ( $F = 85.058$ ;  $d.f. = 11$ ;  $P < 0.001$ ), reaching a maximum of 390 open inflorescences per plant in early April (Fig. 2a). *A. barba-jovis* plants prevented from entomophilous pollination showed inbreeding depression, with a strong decrease in seed-set ( $F = 606.549$ ;  $d.f. = 1$ ;  $P < 0.001$ ) (Fig. 2b). Pollinator abundance reached a maximum in April. The most abundant species were honeybees, *Apis mellifera* (Fig. 3a) and bumblebees (i.e. *Bombus pascuorum* and *Bombus terrestris*) (Table 1). Scanning electron microscopy showed that all pollinator species carried *A. barba-jovis* pollen on their body parts, particularly third-leg femora e.g. pollen

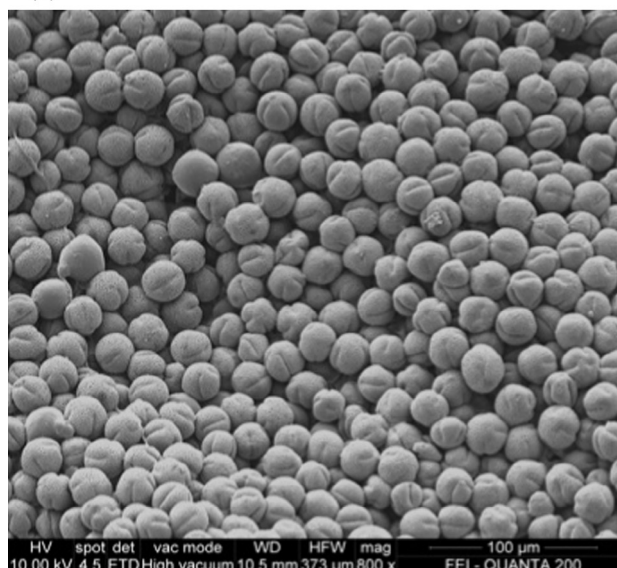


**Fig. 2.** (a) Number of open inflorescences per plant of *Anthyllis barba-jovis* growing in an arid coastal area of Tuscany (Italy) during spring 2015. T-bars indicate standard deviations. Different letters indicate significant differences amongst the number of open flowers per plant over time (one-way ANOVA followed by Tukey's HSD test,  $P < 0.05$ ). (b) Reproductive performances of insect-pollinated (open) and bagged (self) inflorescences of *Anthyllis barba-jovis* in terms of number of produced seeds. Different letters indicate significant differences (one-way ANOVA,  $P < 0.05$ ).

(a)



(b)



**Fig. 3.** (a) A honeybee, *Apis mellifera*, foraging on flowers of *Anthyllis barba-jovis*. (b) Scanning electron micrograph of *A. barba-jovis* pollen mass-packed in the pollen baskets located on the third pair of honeybee legs.

grains mass-packed in the pollen baskets of honeybees (Fig. 3b). Notably, honeybee visits on *A. barba-jovis* flowers decreased from early April to late May, when the blooming was less abundant and more flowering species, including Asteraceae, Cistaceae, Fabaceae and Rhamnaceae become available to bees (Table 2).

## 4. Discussion

Our experiments showed that *A. barba-jovis* was mainly pollinated by social Apoidea, mainly honeybees and bumblebees. As regards the insect pollinators of other species belonging to the genus *Anthyllis*, *A. vulneraria* subsp. *vulgaris* growing in northwest Spain is mainly pollinated by the long-tongued bee *Anthophora acervorum*, which accounted for about 45% of recorded insect visits. In addition, just over 45% of visits are by the nectar-robbing bumblebees *B. terrestris* and *Bombus jonellus* (Navarro, 2000).

According to us, the *A. barba-jovis* production of pollen and nectar in periods characterized by food paucity for insect pollinators in Mediterranean coastal environments, is of particular importance for bumblebees. Indeed, a continuous and readily available supply of food is crucial for their successful establishment and growth



**Table 1**

Total abundance of insect pollinators visiting *Anthyllis barba-jovis* flowers in an arid coastal environment of Tuscany (central Italy) during spring 2015. Different letters indicate significant differences (generalized linear model,  $P < 0.05$ ).

Species	Family	Abundance (n)			
		March	April	May	Total
<i>Anthophora crinipes</i> Smith, 1854	Apidae	1	4	1	6 <sup>c</sup>
<i>Anthophora plumipes</i> Pallas, 1772	Apidae	1	6	0	7 <sup>c</sup>
<i>Apis mellifera</i> (Linnaeus, 1758)	Apidae	18	197	93	308 <sup>a</sup>
<i>Bombus pascuorum</i> (Scopoli, 1763)	Apidae	8	7	1	16 <sup>b</sup>
<i>Bombus terrestris</i> (Linnaeus, 1758)	Apidae	3	11	4	18 <sup>b</sup>
<i>Oxythreya funesta</i> (Poda, 1761)	Scarabaeidae	1	2	1	4 <sup>c</sup>
<i>Xylocopa violacea</i> Linnaeus, 1758	Apidae	1	2	1	4 <sup>c</sup>

Within the total abundance column, different letters indicate significant differences (generalized linear model,  $P < 0.05$ ).

**Table 2**

Appreciable flowering of bee-visited species (by direct observation) during the study period. Observations were carried out in a radius of 500 m from the selected plants of *Anthyllis barba-jovis*.

Species	Family	Month								
		March			April			May		
		Decades								
		1	2	3	1	2	3	1	2	3
<i>Carpobrotus edulis</i> (L.) N.E.Br.	Aizoaceae					●	●	●	●	●
<i>Cistus incanus</i> L.	Cistaceae							●	●	●
<i>Cistus monspeliensis</i> L.	Cistaceae							●	●	●
<i>Cistus salvifolius</i> L.	Cistaceae							●	●	●
<i>Coronilla juncea</i> L.	Fabaceae		●	●	●	●				
<i>Cytisus scoparius</i> (L.) Wimm.	Fabaceae			●	●	●	●	●		
<i>Dorycnium hirsutum</i> (L.) Ser.	Fabaceae						●	●	●	●
<i>Helichrysum italicum</i> L.	Asteraceae									●
<i>Rhamnus alaternus</i> L.	Rhamnaceae	●	●	●						
<i>Senecio cineraria</i> L.	Asteraceae					●	●	●	●	●
<i>Teucrium flavum</i> L.	Lamiaceae								●	●

The dots in the table represents appreciable flowering.

(Goulson et al., 2008). Flower availabilities during colony foundation (early spring) and colony reproduction (mid to late summer) have been suggested as key resource bottlenecks in European landscapes (Rundlöf et al., 2014; Rollin et al., 2016). However, the most frequent insects on flowers of a given plant are not necessarily the most efficient pollinators (Benvenuti et al., 2016; Benelli et al., 2017). Indeed, pollen transport depends on the particular morphology of the insect body parts (e.g. pollen baskets or hairs, and on the speed of insects whilst handling flowers). For instance, the rapid visits performed by long-tongued bees may result in reduced pollen transport (Fenster et al., 2004). However, the electrostatic forces occurring on pollen can guarantee adhesion to the insect, even if it is lacking in hairy structures (Armbruster, 2001). In addition, the time of the observations may have influenced the insect pollinators' abundance and diversity. Therefore, detailed observations in early hours of the day should be considered for future research.

In the last experiment, *A. barba-jovis* plants prevented from entomophilous pollination showed inbreeding depression, with a decrease in seed-set, highlighting the pivotal importance of pollinator visits for seed production, as previously reported for other *Anthyllis* species (Navarro, 1996, 1999). Overall, this research showed the importance of *A. barba-jovis* shrub spots as food for bees in coastal areas of the Mediterranean basin, when alternative food sources are scarce. To the best of our knowledge, this is the first report about insect pollinators of *A. barba-jovis*.

### Conflict of interest

The authors declare no competing interests.

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